VERIFICATION OF TRANSLATION

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[LIST OF DOCUMENTS SUBMITTED]

[TITLE OF DOCUMENT]	Claims	1
[TITLE OF DOCUMENT]	Specification	1
[TITLE OF DOCUMENT]	Drawing	1
[TITLE OF DOCUMENT]	Abstract	1

【TITLE OF DOCUMENT】 CLAIMS

【Claim 1】

An anti-SARS virus monoclonal antibody against nucleoprotein of a corona virus which causes severe acute respiratory syndrome (SARS).

【Claim 2】

The anti-SARS virus monoclonal antibody according to claim 1, which monoclonal antibody is produced by a hybridoma prepared by using as an immunogen the nucleoprotein of said coronavirus, said nucleoprotein being expressed by a vector in which a nucleotide sequence shown in SEQUENCE LISTING 1 is incorporated.

【Claim 3】

The anti-SARS virus monoclonal antibody according to claim 2, which monoclonal antibody has binding specificity of the monoclonal antibody produced by hybridoma rSN-18 having an Accession No. FERM P-19572, hybridoma rSN-122 having an Accession No. FERM P-19573, hybridoma rSN-150 having an Accession No. FERM P-19574, hybridoma rSN-21-2 having an Accession No. FERM P-19619 or hybridoma rSN-29 having an Accession No. FERM P-19620.

【Claim 4】

The monoclonal antibody which is produced by a hybridoma prepared by using as an immunogen a nucleoprotein of said SARS virus shown in SEQUENCE LISTING 3.

【Claim 5】

A hybridoma producing said monoclonal antibody according to any one of claims 1 to 3, which hybridoma is obtained by fusing an anti-SARS virus monoclonal antibody-producing cell and a tumor cell.

【Claim 6】

A hybridoma according to claim 5, which hybridoma is hybridoma rSN-18

having an Accession No. FERM P-19572, hybridoma rSN-122 having an Accession No. FERM P-19573, hybridoma rSN-150 having an Accession No. FERM P-19574, hybridoma rSN-21-2 having an Accession No. FERM P-19619 or hybridoma rSN-29 having an Accession No. FERM P-19620.

【Claim 7】

A reagent for immunoassay of SARS-causing coronavirus, comprising said monoclonal antibody according to any one of claims 1 to 4 as at least one of immobilized antibody and labeled antibody.

【Claim 8】

An immunoassay device comprising a detection zone having an anti-SARS antibody immobilized on a matrix through which liquid can be transported; and a labeled reagent zone on which a labeled anti-SARS antibody is spotted on said matrix in such a manner that said labeled anti-SARS antibody is mobile; at least one of said antibody immobilized on said detection zone and said labeled anti-SARS antibody being said monoclonal antibody according to any one of claims 1 to 4.

【Claim 9】

The immunoassay device according to claim 8, wherein said label is an enzyme and wherein said immunoassay device has a substrate at a region upstream of said labeled reagent zone in said matrix, said substrate reacting said enzyme.

【TITLE OF DOCUMENT】 SPECIFICATION

【TITLE OF THE INVENTION】 Anti-SARS Virus Antibody, Hybridoma Producing the Antibody and Immunoassay Reagent Using the Antibody

【Technical Field】

【0001】

The present invention relates to a monoclonal antibody against the nucleoprotein of the severe acute respiratory syndrome (SARS)-causing coronavirus (hereinafter referred to as "SARS virus"), hybridoma which produces the monoclonal antibody, and to an immunoassay reagent or immunoassay device for SARS virus, which uses the monoclonal antibody as the immobilized antibody and/or labeled antibody.

【Background Art】

【0002】

From 2002 to 2003, patients suffering from severe pneumonia were reported worldwide, and a number of death were reported in addition to the infected patients. The virus isolated from the patients was named SARS virus, and the virus was confirmed to be a new type of coronavirus. The whole genome has been sequenced by Michael Smith Genome Sciences Centre in British Columbia, Canada (Non-patent Literature 1).

After incubation period of 2 to 7 days from the infection by SARS virus, the SARS virus causes high fever higher than 38°C, coughs, headache, dyspnea and so on. Since the symptoms of SARS are similar to those of influenza, diagnosis whether the infection is by SARS or not at an early stage is demanded. Reported diagnoses of infection by SARS virus include the following:

1) Measurement of Antibody by ELISA: Antibodies (IgM/IgA) in sera of SARS patients may be detected after about 20 days from the manifestation of clinical symptoms.

2) Immunofluorescence Method: Immunofluorescence method using VERO cells infected with SARS virus (detecting IgM). Antibody in serum may be detected after about 10 days from the onset.

3) PCR Method: SARS virus gene products from various specimens such as blood, feces and respiratory secretions are amplified and detected.

4) Cell Culture Method: Virus in a specimen from a SARS patient is infected to culture cells such as VERO cells and then detected.

【0003】

【Non-patent Literature 1】 Science; 2003 May 30;300(5624):1394-9

【Disclosure of the Invention】

【Problems Which the Invention Tries to Solve】

【0004】

Among the known methods for confirming infection by SARS virus, with the antibody test methods, the infection can be detected only after 10 days from the infection, and the highly reliable immunofluorescence method is complicated. As for the PCR method, since it is necessary to isolate and amplify a SARS-related gene, the method requires a special amplification apparatus and measurement apparatus, and is not a simple measurement method. As for the cell culture method, it is difficult to process a number of specimens, and infection by SARS virus cannot be confirmed only by this method, even though the infection by coronavirus may be confirmed.

In view of the above-described circumstances, an object of the present invention is to provide a monoclonal antibody which specifically recognizes SARS virus, and to provide an immunoassay reagent or immunoassay device for detecting SARS virus which utilizes the monoclonal antibody.

【Means to Solve the Problems】

【0005】

The present inventors intensively studied for obtaining an anti-SARS virus monoclonal antibody having specificity to SARS virus and having a high affinity to obtain the desired monoclonal antibody by obtaining a nucleoprotein gene of SARS virus from synthetic nucleotide synthesized by utilizing PCR; preparing a transformant using the obtained gene in accordance with gene manipulation techniques; and using, as an immunogen, the nucleoprotein of SARS virus obtained from the transformant. Further, the present inventors were able to develop an immunoassay reagent using the monoclonal antibody.

【0006】

The monoclonal antibody according to the present invention is a monoclonal antibody which specifically recognizes the nucleoprotein of SARS virus and which is produced by a hybridoma prepared by using as an immunogen the nucleoprotein of the above-described coronavirus, said nucleoprotein being expressed by a vector in which a nucleotide sequence shown in SEQUENCE LISTING 1 is incorporated. The present invention is a monoclonal antibody which has binding specificity of the monoclonal antibody produced by hybridoma rSN-18 having an Accession No. FERM P-19572, hybridoma rSN-122 having an Accession No. FERM P-19573, hybridoma rSN-150 having an Accession No. FERM P-19574, hybridoma rSN-21-2 having an Accession No. FERM P-19619, hybridoma rSN-29 having an Accession No. FERM P-19620 or hybridoma SN5-25. Further, the present invention is a hybridoma which produces said monoclonal antibody. Further, the present invention is an immunoassay reagent for measuring the nucleoprotein of SARS virus by reacting said monoclonal antibody with a sample and measuring formed immune complex, and an immunoassay device comprising a detection zone having an anti-SARS antibody immobilized on a matrix through which liquid can be transported; and a labeled reagent zone on which a labeled anti-SARS antibody is spotted on said matrix in such a manner that said labeled anti-SARS antibody is mobile; at least one

of said antibody immobilized on said detection zone and said labeled anti-SARS antibody being the above-described monoclonal antibody.

【Effect of the Invention】

【0007】

Since the monoclonal antibody according to the present invention has a high specificity and high reactivity to the nucleoprotein of SARS virus, the monoclonal antibody may be used for highly sensitive immunoassay of SARS virus. The hybridoma according to the present invention can provide a monoclonal antibody which specifically recognizes SARS virus. Further, the immunoassay reagent utilizing the monoclonal antibody according to the present invention may detect only the samples containing SARS virus or only the samples from SARS patients by simple operations.

【Best Mode for Carrying out the Invention】

【0008】

The present invention will now be described in detail. The monoclonal antibody according to the present invention is a monoclonal antibody which specifically reacts with the nucleoprotein of SARS virus (hereinafter occasionally referred to as "anti-SARS virus monoclonal antibody"). To obtain the monoclonal antibody according to the present invention, the nucleoprotein, preferably the nucleoprotein produced in accordance with gene manipulation or chemical synthesis, may be used as an immunogen. As the recombinant antigen, the polypeptide substantially containing the amino acid sequence shown in SEQ ID NO:2 in SEQUENCE LISTING may be used. As the chemically-synthesized nucleoprotein, for example, the polypeptide shown in SEQ ID NO:3 may be used.

【0009】

In the present invention, a monoclonal antibody having high specificity to the nucleoprotein of SARS virus may be obtained by using as an immunogen the above-

described polypeptide substantially containing the amino acid sequence shown in SEQ ID NO:2 in SEQUENCE LISTING.

【0010】

As the nucleoprotein of SARS virus used as the above-described immunogen, any polypeptides may be available as long as the polypeptides substantially contain the amino acid sequence shown in SEQ ID NO:2 in SEQUENCE LISTING, and a polypeptide containing the amino acid sequence shown in SEQ ID NO:2 to which other peptide(s) is(are) attached to back and/or forth to the extent that the property as an immunogen is not adversely affected may be used as an immunogen. Alternatively, whole region of the nucleoprotein of SARS virus may also be used as an immunogen. The nucleoprotein may not be necessarily highly purified, and crudely purified nucleoprotein may also be used as an immunogen. "Polypeptides substantially containing the amino acid sequence shown in SEQ ID NO:2 in SEQUENCE LISTING" means that the polypeptides include those containing the same amino acid sequence shown in SEQ ID NO:2 except that one or several amino acids in the amino acid sequence shown in SEQ ID NO:2 are deleted, substituted and/or added to the extent that the function or three-dimensional structure of the nucleoprotein of SARS virus is not adversely affected.

【0011】

The nucleoprotein of SARS virus used as the above-described immunogen may be obtained by, for example, the following method using gene manipulation technique:

By amplifying the gene region encoding the nucleoprotein of SARS virus by PCR and cleaving the product by restriction enzyme, a DNA fragment (SEQUENCE LISTING 1) of the region encoding the above-described polypeptide substantially containing the amino acid sequence shown in SEQ ID NO:2 in SEQUENCE LISTING is obtained. Alternatively, a DNA fragment of the region encoding the

polypeptide substantially containing the amino acid sequence shown in SEQ ID NO:2 or NO:3 in SEQUENCE LISTING may be chemically synthesized based on the above-described nucleotide sequence. The thus obtained DNA fragment may be incorporated into an expression vector having an appropriate marker gene such as ampicillin-resistant gene, and a host such as *E. coli* may be transformed with the resulting recombinant DNA to obtain a transformant. By culturing the obtained transformant and by purification of the culture medium, the above-described nucleoprotein of SARS virus may be obtained. Polypeptides such as those containing the sequence shown in SEQ ID NO:3 may also be obtained as the nucleoprotein by a known synthesis method using a synthesizer.

【0012】

The above-described anti-SARS virus monoclonal antibody may be produced by a hybridoma obtained by immunizing an animal with the polypeptide substantially containing the amino acid sequence shown in SEQ ID NO:2 or NO:3 in SEQUENCE LISTING, and fusing anti-nucleoprotein of SARS virus antibody-producing cells obtained from the animal and tumor cells.

【0013】

The above-described hybridoma may be obtained by the following method: That is, the nucleoprotein of SARS virus obtained as described above is intrapectoneally or intravenously administered to an animal such as mouse together with Freund's complete adjuvant, dividedly in several times, at 2 to 3-week intervals, thereby immunizing the animal. Then the antigen-producing cells originated from the spleen or the like and tumor cells which can proliferate *in vitro* such as cells originated from myeloma cell line (myeloma cells) are fused.

【0014】

The above-described fusion may be attained by using polyethylene glycol in accordance with the conventional method by Kohler and Milstein (Nature, Vol.256,

page 495, 1975), or by using Sendai virus or the like.

【0015】

Selection of hybridomas producing the antibody which recognizes the nucleoprotein of SARS virus from the fused cells may be attained by the following method: That is, cells which are alive in HAT medium may be selected as hybridomas from the fused cells. Then the culture medium of each of the obtained hybridomas may be reacted with highly purified nucleoprotein of SARS virus immobilized on an assay plate, and thereafter the assay plate may be reacted with anti-mouse immunoglobulin (Ig) or the like. By such an EIA, hybridomas producing monoclonal antibodies which specifically recognize the nucleoprotein of SARS virus may be selected.

【0016】

The hybridoma according to the present invention is not restricted as long as it produces a monoclonal antibody which specifically recognizes the nucleoprotein of SARS virus. Examples of the hybridoma include the 6 hybridomas established by the above-described method by the present inventors.

【0017】

The 6 hybridomas were named hybridoma rSN-18, hybridoma rSN-122, hybridoma rSN-150, hybridoma rSN-21-2, hybridoma rSN-29 and hybridoma SN5-25, respectively.

【0018】

These hybridomas have been deposited with International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology (address: 1-1-1 Higashi, Tsukuba, Ibaraki, Japan). That is, hybridoma rSN-18 has been deposited under Accession No. FERMP-19572 (date of receipt: October 24, 2003), hybridoma rSN-122 has been deposited under Accession No. FERMP-19573 (date of receipt: October 24, 2003), hybridoma rSN-150 has been deposited under

Accession No. FERMP-19574 (date of receipt: October 24, 2003), hybridoma rSN-21-2 has been deposited under Accession No. FERMP-19619 (date of receipt: December 26, 2003) and hybridoma rSN-29 has been deposited under Accession No. FERMP-19620 (date of receipt: December 26, 2003).

【0019】

Each of the above-described hybridoma may be cultured in a culture medium ordinarily used for cell culture. The monoclonal antibody may be recovered from the culture supernatant. Alternatively, the above-described hybridoma may be administered to an animal of the same species as the animal from which the hybridomas were derived to accumulate ascites therein, and the monoclonal antibody may be recovered from the ascites.

【0020】

The monoclonal antibody may be recovered by a purification method conventionally employed. Examples of the purification method include gel permeation chromatography, ion-exchange chromatography and affinity chromatography using protein A.

【0021】

The reactivity of the monoclonal antibody may be confirmed by a usual method. The monoclonal antibody of the present invention is a monoclonal antibody which specifically recognizes the nucleoprotein of SARS virus.

【0022】

The reagent for immunoassay of SARS virus according to the present invention may be produced by using the above-described monoclonal antibody as at least one of the solid phase antibody and labeled antibody. As the solid phase on which the above-described monoclonal antibody is immobilized, various solid phases used in conventional immunoassays may be used. Examples of such solid phases include various solid phases such as ELISA plates, latices, gelatin particles, magnetic

particles, polystyrenes and glasses, insoluble carriers such as beads and matrices through which liquid can be transported and the like. The labeled reagent may be produced by labeling an antibody with an enzyme, colloidal metal particle, colored latex particle, luminescent substance, fluorescent substance, radioactive substance or the like. By combining these reagents, reagents used in enzyme immunoassays, radioimmunoassays, fluoroimmunoassays or the like may be produced. These measurement reagents are the reagents for measuring an antigen of interest present in the test sample by sandwich immunoassay or competitive binding immunoassay. The immunoassay device for SARS virus, which utilizes the principle of immunochromatography, comprising a detection zone having a monoclonal antibody of the present invention immobilized on a matrix through which liquid can be transported; and a labeled reagent zone on which a labeled anti-SARS virus monoclonal antibody of the present invention is spotted on said matrix in such a manner that said labeled antibody is mobile may also be produced.

【0023】

A reagent for the above-described sandwich immunoassay may be provided by, for example, providing two monoclonal antibodies according to the present invention, and using one of them as the above-described labeled antibody and using the other as the immobilized antibody bound to the solid phase. First, the solid phase reagent is reacted with a sample containing an antigen to be measured, and then the bound antigen is reacted with the labeled monoclonal antibody (second antibody). By determining the amount of the antigen to be measured based on the amount of the label, that is, labeled antibody, bound to an insoluble carrier, immunoassay may be attained. As reagents for immunoassay used in sandwich immunoassay, although one type of monoclonal antibody may be used as both of the solid phase antibody and the labeled antibody, it is usually preferred to use antibodies recognizing two different epitopes contained in the antigen to be measured and to

select a solid phase antibody and a labeled antibody respectively from different monoclonal antibodies. Further, as the solid phase antibody and as the labeled antibody, respectively, a plurality of monoclonal antibodies selected from two or more types of monoclonal antibodies may be used in combination.

【0024】

As a reagent for immunoassay used in competitive binding immunoassay, for example, a certain amount of a virus antigen labeled with an enzyme, colloidal metal particle, colored latex particle, luminescent substance, fluorescent substance, radioactive substance or the like is prepared. Using this reagent, for example, a certain amount of the monoclonal antibody of the present invention, the above-described labeled virus antigen and a sample containing the antigen to be measured may be reacted competitively, and the amount of the antigen to be measured may be determined based on the amount of the labeled virus antigen bound or not bound to the antibody, thereby attaining immunoassay. To separate the labeled antigen bound to the antibody from the one which is not bound to the antibody, the double antibody method in which immunoglobulin of the same species as the antibody and an antibody against the immunoglobulin are added and the labeled antigen bound to the antibody is precipitated to be measured, the method using charcoal or millipore filter or the like may be employed.

【0025】

In the present invention, a labeled antibody or labeled antigen may be prepared in accordance with a method such as physical adsorption or chemical bond in order to bind the above-described antibody or antigen to the solid phase or the label (see "PROTEIN, NUCLEIC ACID AND ENZYME", Extra Edition, vol.31, pp.37-45 (1987))

【0026】

By utilizing the anti-SARS virus monoclonal antibody of the present

invention for an immunoassay device which utilizes the principle of immunochromatography, SARS virus present in a sample may be detected easily without using a special measuring apparatus. This immunoassay device comprises a belt-like matrix through which liquid can be transported by capillary action, which matrix comprises a SARS virus detection zone on which at least one type of anti-SARS virus monoclonal antibody is immobilized; a labeled reagent zone on which a labeled anti-SARS virus monoclonal antibody is spotted movably; a sample-spotting zone; a developer-supply zone having a developer pad mounted at one end of the above-described matrix in the longitudinal direction; and a developer-absorption zone formed at the other end of the above-described matrix in the longitudinal direction.

【0027】

The matrix in this immunoassay device is made of a belt-shaped, water-absorptive material in which liquid can be transported by capillary action. Examples of the water-absorptive material include cellulose and derivatives thereof such as cellulose and nitrocellulose, and filter papers made of glass fibers alone or containing glass fibers, membranes and porous materials. Although the size of the matrix is not restricted, those in the form of strip having a width of about 3 mm to 10 mm, and a length of about 30 mm to 100 mm are preferred because they have good ease of handling. The thickness of the matrix may be 100 μm to 1 mm. To prevent non-specific adsorption of the proteins originated from the sample to the matrix during the measurement, a part or the entire matrix may be blocked with an animal serum protein such as bovine serum albumin (BSA), casein, sucrose or the like.

【0028】

(Detection Zone)

In the detection zone, a SARS virus-detection section in which the anti-SARS

virus monoclonal antibody is immobilized on the matrix may be provided. At least one of this anti-SARS virus monoclonal antibody immobilized in the detection section and the labeled anti-SARS virus monoclonal antibody hereinbelow described is the anti-SARS virus monoclonal antibody according to the present invention, and preferably, both of them are the anti-SARS virus monoclonal antibody. The anti-SARS virus monoclonal antibody in the detection section is preferably arranged on the matrix, in the form of a line perpendicular to the direction of the flow of the liquid (longitudinal direction of the matrix) for attaining the measurement with high sensitivity.

【0029】

The anti-SARS virus monoclonal antibody in the detection zone is the above-described antibody, and the monoclonal antibody may be used individually or a plurality of the antibodies may be used in combination. The anti-SARS virus monoclonal antibody may be IgG antibody or IgM antibody, or may be Fab, Fab', $F(ab')_2$ or the like which is an fragment of these antibodies.

【0030】

The anti-SARS virus antibody immobilized in the detection section may be physically adsorbed directly in the detection zone on the matrix, or may be fixed by chemical bond such as covalent bond. Alternatively, the anti-SARS virus monoclonal antibody may be bound to a water-insoluble carrier and the carrier may be incorporated in the matrix. Examples of the insoluble carriers include the particles obtained by insolubilizing a mixture of gelatin, gum arabic and sodium hexametaphosphate (Japanese Patent Publication (Kokoku) No. 63-29223), polystyrene latex particles, glass fibers and the like. The anti-SARS virus monoclonal antibody may be bound to the insoluble carrier by the above-described chemical bond or by physical adsorption.

【0031】

On the matrix, the detection section is located downstream of the labeled reagent zone, the sample-spotting zone and the developer-supply zone in the direction of the flow of the developer, and located upstream of the developer-absorption zone. The detection section may be arranged on the matrix in the form of closely-aligned multiple lines having a total width of about 0.5 mm to 5 mm. In the case of a matrix having a width of about 5 mm, the detection section may be formed by spotting the above-described antibody and antigen usually in an amount of about 0.1 μg to 10 μg, respectively, and drying the matrix.

【0032】

(Labeled Reagent Zone)

The labeled reagent zone may be formed on the matrix by spotting a labeled anti-SARS virus monoclonal antibody movably. This zone may be formed upstream of the above-described detection zone in the direction of the flow of the developer from the developer-supply zone. This zone may be formed by spotting the labeled reagent on the matrix, by laminating a water-absorptive pad containing the labeled reagent, or by incorporating the labeled reagent in a part or the entire region of the matrix which intimately contacts the pad. As the water-absorptive pad, the pad used for the sample-spotting zone hereinbelow described may be used.

【0033】

At least one of the labeled anti-SARS virus monoclonal antibody and the antibody immobilized in the detection zone is the anti-SARS virus monoclonal antibody, and both of them are preferably the anti-SARS virus monoclonal antibody. As the labeled anti-SARS virus monoclonal antibody, fragments thereof may be employed as in the case of the above-described antibody in the detection zone.

【0034】

The labeled anti-SARS virus monoclonal antibody may be prepared by binding the above-described antibody with the label. Examples of the label include

enzymes, colloidal metal particles, colored latex particles, fluorescent latex particles, luminescent substances, fluorescent substances and the like. As the enzyme, various enzymes used in enzyme immunoassays (EIA) may be employed. Examples of the enzyme include alkaline phosphatase, peroxidase, β-D-galactosidase and the like. Examples of the colloidal metal particles include colloidal gold particles, colloidal selenium particles and the like.

【0035】

The known methods using covalent bond or non-covalent bond may be used for binding the label and the anti-SARS virus monoclonal antibody. Examples of the binding method include glutaraldehyde method, periodate method, maleimide method, pyridyl disulfide method and methods using various cross-linking agents (see, for example, "PROTEIN, NUCLEIC ACID AND ENZYME", Extra Edition, vol.31, pp.37-45 (1985)). In the methods using a cross-linking agent, for example, *N*-succinimidyl-4-maleimide butyric acid (GMBS), *N*-succinimidyl-6-maleimide hexanoic acid, *N*-succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylic acid or the like may be used as a cross-linking agent. In the methods using covalent bond, the functional groups existing in the antibody may be used. Alternatively, the labeled anti-SARS virus monoclonal antibody may be prepared by binding the label to the functional group using the above-described binding method after introducing a functional group such as thiol group, amino group, carboxyl group, hydroxyl group or the like by a conventional method. In the methods using non-covalent bond, the physical adsorption or the like may be used.

【0036】

Although the amount of the labeled anti-SARS virus monoclonal antibody may be appropriately selected depending on the expected amount of the test substance in the sample, it is usually about 0.01 μg to 5 μg in terms of dry weight. The labeled anti-SARS monoclonal antibody may be applied together with a

stabilizer, solubilization-adjusting agent or the like.

【0037】

(Sample-spotting Zone)

The sample-spotting zone may be formed in the matrix at a site downstream of the developer-supply zone and upstream of the detection zone in the direction of the flow of the developer, without incorporating a reagent or the like. The sample-spotting zone may also be formed at 1) a prescribed site downstream of the developer-supply zone and upstream of the labeled-reagent zone in the direction of the flow of the developer, 2) a prescribed site downstream of the labeled reagent zone and upstream of the detection zone in the direction of the flow of the developer, or 3) a prescribed site on the labeled reagent zone. In the device in which the sample-spotting zone is formed in the labeled reagent zone, it is preferred to add the water-absorptive pad containing the labeled reagent for carrying out the assay efficiently as mentioned above. By using the device comprising the pad, since a large amount of sample fluid may be spotted, a minor component in the sample may be measured with high detection sensitivity. The material constituting the water-absorptive pad is selected from the materials which scarcely adsorb the labeled reagent and the SARS virus in the sample. Examples of such materials include porous materials made of synthetic or natural macromolecular compounds such as polyvinyl alcohol (PVA), non-woven fabric, cellulose and the like, and these materials may be employed individually or in combination. Although the size, thickness, density and the like of the pad are not restricted, it is usually preferred to use a pad having longitudinal and lateral lengths of about 3 mm to 10 mm, and a thickness of about 0.5 mm to 4 mm for carrying out the assay efficiently.

【0038】

(Developer-supply Zone)

The developer-supply zone is the zone formed at one end of the matrix in the

longitudinal direction, to which the developer is supplied. The assay may be started by immersing this zone in the developer contained in a vessel in an amount at least sufficient to reach the developer-absorption zone. A liquid bath containing the developer may be attached to the developer-supply zone, and the assay may be started by breaking a cover of the liquid bath thereby bringing the developer into contact with the matrix. The developer may appropriately contain a surfactant, buffering agent, stabilizer, antibacterial agent or the like. In cases where an enzyme is used as the label, the substrate may be added to the developer in addition to the substrate zone hereinbelow described. Examples of the buffer solution containing a buffer agent include acetate buffer, borate buffer, Tris-HCl buffer, diethanolamine buffer and the like. On the developer-supply zone, a developer pad may be mounted to stably and continuously supply the developer to the matrix. As the developer pad, a filter paper made of, for example, cellulose or cellulose derivative may be employed.

【0039】

(Developer-absorption Zone)

The developer-absorption zone is provided in the matrix at the end other than the end at which the above-described developer-supply zone is provided. This zone is provided for absorbing the developer supplied to the matrix so as to fluently carry out the assay. The developer-absorption zone may be provided by elongating the matrix. Alternatively, the absorption zone may be provided by arranging a water-absorptive material on the matrix to accelerate the development of the developer. As the water-absorptive material, filter papers having a high water holding capacity, made of natural macromolecular compounds, synthetic macromolecular compounds or the like, or sponge or the like may be employed. The developer-absorption zone is constituted of an absorptive material in the form of a pad having a volume enough to absorb the whole developer. By laminating the absorptive material on or below the matrix, a compact immunoassay device may be produced.

【0040】

(Substrate Reagent Zone)

In cases where an enzyme is used as the label contained in the labeled reagent zone, the substrate may be contained in the developer as mentioned above, or a substrate reagent zone may be provided in the matrix in the vicinity of the developer-supply zone. The substrate reagent zone may preferably be formed in the above-described developer pad mounted on the developer-supply zone by incorporating the substrate in the developer pad for increasing the amount of the substrate so as to carry out the assay with high sensitivity.

【0041】

As the substrate, various coloring substrates, fluorescent substrates, luminescent substrates or the like described below may be used depending on the enzyme in the labeled reagent.

【0042】

(a) Coloring Substrates

For Peroxidase: 2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonate) (ABTS), 3,3',5,5'-tetramethylbenzidine (TMB) and diaminobenzidine (DAB), each in combination with hydrogen peroxide;

For Alkaline Phosphatase: 5-bromo-4-chloro-3-indolyl phosphate (BCIP), p-nitrophenyl phosphate (p-NPP) and 5-bromo-4-chloro-3-indolyl phosphate disodium salt (BCIP·Na)

(b) Fluorescent Substrates

For Alkaline Phosphatase: 4-methylumbelliferyl phosphate (4MUP)

For β-D-galactosidase: 4-methylumbelliferyl-β-D-galactoside (4MUG)

(c) Luminescent Substrates

For Alkaline Phosphatase: 3-(2'-spiroadamantan)-4-methoxy-4-(3"-phosphoryloxy)phenyl-1,2-dioxetane·2 sodium salt (AMPPD)

For β-D-galactosidase: 3-(2'-spiroadamantan)-4-methoxy-4-(3"-β-D-galactopyranosyl)phenyl-1,2-dioxetane (AMGPD)

For Peroxidase: luminol and isoluminol, each in combination with hydrogen peroxide.

【0043】

In cases where the substrate is provided as the substrate zone, the substrate zone may usually be formed by applying an aqueous solution of the substrate in the form of a line on the developer pad, and drying the solution. If desired, a signal-increasing agent, stabilizer, solubilization-adjusting agent or the like may be added. The site of the substrate zone is not restricted as long as it is within the developer pad mounted on the end of the matrix. The amount of the substrate added to the developer or the developer pad may be selected depending on the assay conditions, and may usually be about 5 μg to 500 μg per device.

【0044】

(Method for Using Immunoassay Reagent)

With the immunoassay reagent according to the present invention, SARS virus in various samples may be assayed. The assay may be carried out by first supplying a sample to the sample-spotting zone of the immunoassay device of the present invention, and then supplying the developer to the developer pad, thereby developing the sample in the matrix. The developer moves in the matrix by capillary action to reach the developer-absorption zone. The components in the sample, which have not been bound to the detection zone, the enzyme-labeled reagent and the like, are absorbed by the developer-absorption zone, and the development is completed. After a prescribed time (usually 10 minutes to 20 minutes), the detection zone is observed, and the label bound to the detection section by the SARS virus in the sample is measured, thereby measuring the SARS virus. The detection may be carried out by visual observation or by using a measuring

device such as colorimeter, fluorophotometer, photon counter, photosensitive film or the like, depending on the label or depending on each of the label and the enzyme employed. For the measurement, the method in which the coloring of the detection zone is visually observed, for example, is simple. By this method, by using a color chart corresponding to the concentration of the SARS virus, a semiquantitative assay may be attained. Quantification may also be attained by digitizing the coloring of the detection zone by a colorimeter or the like.

【0045】

The matrix may be laminated and fixed on a support made of a plastic, metal, paper or the like. By fixing the matrix in a case made of a plastic or the like, providing a bath containing the developer in the developer-supply zone, and covering the matrix with a case having through holes at the sites of each zone described above, a device having a good ease of handling may be constituted.

【0046】

As the sample to be assayed by the above-described reagent is not restricted as long as it contains the nucleoprotein of SARS virus. Examples of the samples include sera, plasma and whole blood from human and animals; body fluid extracts such as nasal swab, nasal aspirate and throat swab; respiratory secretion, cell homogenates and tissue homogenates. These samples in the form of solutions containing SARS virus may be used as they are. Alternatively, solutions containing the virus treated with a surfactant such as nonionic surfactant, anionic surfactant or the like may be used. Examples of the nonionic surfactant include Nonidet (Nonidet T-40), Triton and Brij; and examples of anionic surfactant includes SDS.

【0047】

The nucleoprotein of SARS virus distributed in cells, tissues or the like may also be directly measured by fixing the various cells, tissues and the like originated from human or animals, and reacting the monoclonal antibody according to the

present invention therewith. Further, the so called Western blotting, affinity chromatography or the like may be carried out using the monoclonal antibody according to the present invention.

【0048】

By applying the measurement method of the nucleoprotein of SARS virus using the monoclonal antibody according to the present invention to various samples from human or animals, diagnosis of infection by SARS virus may be carried out. By using the monoclonal antibody according to the present invention, the nucleoprotein of SARS virus in various body fluids, cells, tissues and the like from human or animals may be directly measured by immunochemical or immunohistochemical method. It is suspected that SARS virus infected from mammals, birds or the like to human. Thus, in addition to the measurement of human samples, by measuring animal samples, the monoclonal antibody according to the present invention may also be used for the clarification of infection route.

【Examples】

【0049】

The present invention will now be described by way of Reference Examples and Examples. However, the present invention is not restricted to the following Examples.

【0050】

Reference Example 1 Construction of Plasmid

Full length nucleoprotein gene (referred to as "*N*") consists of 1270 base pairs. Based on the reported gene sequence, oligomers of 50 to 55 bases having an overlapping of 15 bases each other were prepared. The *N* gene was divided into two fragments at the restriction site of *Nhe*I which hydrolyzes the *N* gene at about the center thereof, and each fragments was amplified sequentially by PCR. The PCR was performed by using, for the former half fragment, an end primer having an

*Eco*RI site at its 5'-end region, and, for the latter half fragment, an end primer having a *Bam*HI site at its 3'-end region.

After purifying the resulting fragments by PCR Purification Kit from QIAGEN, the former half fragment was hydrolyzed with *Eco*RI and *Nhe*I, and the latter half fragment was hydrolyzed with *Nhe*I and *Bam*HI. The obtained fragments were inserted into the *Eco*RI-*Bam*HI site of an expression plasmid pW6A shown in Fig. 1 to prepare a plasmid pWS-N. *E. coli* BL21 (DE3) (obtained from Brookhaven National Laboratory) was transformed with the obtained plasmid to obtain an ampicillin-resistant transformant *E. coli* BL21(DE3)/pWS-N. The nucleotide sequence and amino acid sequence of the nucleoprotein are shown in SEQUENCE LISTING 1 and 2, respectively.

【0051】

Reference Example 2 Expression of Recombinant Protein (S-N)

The transformant prepared in Reference Example 1 was cultured in 2 ml of LB medium containing 50 μg/ml of ampicillin at 37°C. After growing the transformant in a preliminary culture until the OD of the culture medium reached to 0.6 to 0.8, IPTG was added to a concentration of 0.4 mM to induce the expression, and the culture was continued for another 3 hours. After recovering the bacterial cells by centrifugation of 1.5 ml of culture medium at 5000 rpm for 2 minutes, the cells were suspended in 100 μl of buffer (10 mM Tris-HCl, pH8.0, 0.1 M sodium chloride, 1 mM EDTA), and the suspension was subjected to sonication for 15 minutes to completely disrupt the cells. The thus obtained product was used as the bacterial cell sample.

To 8 μl of the bacterial cell sample, 4 μl of 3 x SDS polyacrylamide buffer (0.15 M Tris-HCl, pH6.8, 6%SDS, 24% glycerol, 6mM EDTA, 2% 2-mercaptoethanol, 0.03% bromphenol blue) was added, and the resulting mixture was subjected to SDS-polyacrylamide gel electrophoresis. The sample was transferred

to a nitrocellulose filter by Western blotting, and the filter was subjected to blocking with 1% BSA, followed by reacting the resulting filter with the monoclonal antibody N5 1000-fold diluted with phosphate buffer (10 mM phosphoric acid, pH7.4, containing 0.15 M sodium chloride). The resulting filter was then reacted with peroxidase-labeled anti-mouse Ig rabbit polyclonal antibody (produced by DAKO), and, after washing, 10 ml of substrate coloring solution (0.01% aqueous hydrogen peroxide solution, 0.6 mg/ml 4-chloro-1-naphthol) was added, thereby coloring the filter. The results are shown in Fig. 2.

【0052】

Reference Example 3 Purification of Soluble S-N

The *E. coli* BL21(DE3)/pWS-N prepared in Reference Example 1 was cultured in LB medium containing ampicillin at 37°C. The transformant was grown in a preliminary culture until a cell population in terms of OD at 600 nm reached to about 0.7, and IPTG was added to 0.4 mM, thereby inducing the expression. After culturing for 18 hours, *E. coli* was recovered by centrifugation. To the recovered *E. coli*, 20 mM Tris-HCl, pH 8.0, 1 mM PMSF (phenylmethylsulfonyl fluoride) was added, and the resulting mixture was sonicated while cooling the mixture on ice. After the centrifugation, ammonium sulfate was added to the soluble fraction S-N, and 20-40% fraction was recovered. This ammonium sulfate fraction was applied to SP Sepharose First Flow (produced by AMERSHAM) equilibrated with 20 mM phosphate buffer, pH 6.9, containing 0.1 M sodium chloride and 8M urea, and eluted with 20 mM phosphate buffer, pH 6.9, containing 0.2 M sodium chloride and 8M urea, thereby carrying out purification. The eluted fraction was dialyzed against 20 mM Tris-HCl buffer, pH8.0, containing 0.2M sodium chloride. The obtained product was subjected to SDS-polyacrylamide gel electrophoresis and Western blot as in Reference Example 2, thereby confirming the degree of purification. As a result, a single band was shown.

【0053】

Example 1 Establishment of Anti-N Protein Monoclonal Antibodies

Anti-N protein monoclonal antibodies were prepared by immunizing mice with the recombinant N protein prepared in Reference Example 3, and fusing the lymphocytes from the spleen of the mice and myeloma cells. That is, BALB/C mice were first immunized with the recombinant N protein emulsified with Freund's complete adjuvant in an amount of 50 to 100 μg/mouse, and 2 to 3 weeks later, second immunization was performed with the same antigen emulsified with Freund's incomplete adjuvant in an amount of 50 to 100 μg/mouse. The antibody titer was checked by solid phase ELISA using a 96-well ELISA plate coated with the recombinant N protein. To the mice in which the raise of the antibody titer was observed, free recombinant N protein was intravenously administered in an amount of 25 to 100 μg. Three to four days later, spleen was removed from each mouse and spleen cells were separated. The obtained spleen cells were mixed with mouse myeloma cells (P3U1) preliminarily cultured in RPMI-1640 medium at a mixing ratio of 1:2 to 1:5, and cell fusion was performed using PEG (produced by Boehringer). The fused cells were suspended in HAT medium and dividedly applied to a 96-well culture plate, followed by incubation at 37°C in a CO_2 incubator.

【0054】

The screening was carried out by the above-described solid phase ELISA. More particularly, a solution of the recombinant N protein with a concentration of 1 μg/ml was added to a 96-well ELISA plate (produced by PHARMACIA) in an amount of 50 μl/well, and the plate was left to stand overnight at 4°C, thereby adsorbing the recombinant N protein to the wells. Each well was blocked with 1% skim milk and washed three times with washing buffer (PBS containing 0.05% Tween 20). To each well, 50 μl of the supernatant of the culture medium in which cell fusion was performed was added, and the resultant was allowed to react at 37°C

for 1 hour. Each well was then washed 3 times with the washing buffer in the same manner as described above, and POD-labeled anti-mouse immunoglobulin antibody (produced by DAKO) was added, followed by allowing the mixture to react at 37°C for 1 hour. After washing the wells 4 times with the washing buffer, the substrate ABTS was added, and the wells which colored were selected. The cells in the selected wells were transferred to a 24-well culture plate and cultured in a CO_2 incubator at 37°C, and the cells were cloned by the limiting dilution method to establish 5 hybridomas which produce the anti-N protein monoclonal antibodies described below, that is, hybridomas rSN-18, rSN-122, rSN-150, rSN-21-2 and rSN-29. These hybridomas have been deposited with the above-described International Patent Organism Depositary under the Accession Nos. FERM P-19572, FERM P-19573, FERM P-19574, FERMP-19619 and FERM P-19620, respectively.

【0055】

Example 2 Confirmation of Reactivities of Monoclonal Antibodies by Western Blotting (WB)

The reactivity of each of the established monoclonal antibodies to the naturally occurring antigen (the N protein originated from the virus) was confirmed by WB using a concentrated virus suspension as a sample. Vero E6 cells were infected with SARS virus strain Hanoi, and the cells were cultured in a CO_2 incubator for 48 hours, followed by centrifugation of the culture medium at 2000 rpm for 15 minutes to prepare a culture supernatant ($TCID_{50}$ was 7.95×10^6/ml). The culture supernatant was inactivated at 56°C for 90 minutes, and then 31.5 ml aliquot thereof was centrifuged at 30Krpm for 3 hours using a Hitachi ultracentrifuge (40T rotor). To the obtained precipitate, TNE (Tris-NaCl-EDTA) buffer (0.3 ml) was added, and pipetting was performed to prepare a concentrated virus suspension. To this suspension, an equivolume of sample-treating solution for electrophoresis was added, and the resulting mixture was heated to obtain a test sample. After

conducting SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12.5% gel, the sample was transferred to a nitrocellulose membrane to prepare a transferred membrane for WB. After blocking the transferred membrane with skim milk, the membrane was subjected to reaction with each of the antibodies. WB was performed using as the anti-N protein monoclonal antibodies, rSN-18 antibody, rSN-122 antibody, rSN-150 antibody, rSN-29 antibody, rSN-21-2 antibody and rSN-122 antibody, and using as a negative control, an unrelated monoclonal antibody E2CT-38 antibody.

The reaction with the antibody was performed as follows: That is, each monoclonal antibody was shaken with the antigen-transferred WB membrane at room temperature for 1 hour, thereby allowing the reaction, and the membrane was washed 3 times (washing under shaking for 5 minutes) with a washing buffer (PBS containing 0.05% Tween 20). Then a POD-labeled anti-mouse immunoglobulin antibody (produced by DAKO) was added, and the reaction was carried out for another 1 hour at room temperature. After washing 4 times (washing under shaking for 5 minutes) with the washing buffer, a substrate 4-chloronaphthol solution was added, and the bands were observed. As shown in Figs. 3 and 4, a band at a position of a molecular weight of little less than 50 Kd corresponding to the N protein was observed when each of the monoclonal antibodies was used.

【0056】

Example 3 Detection of N Protein in Virus Culture Supernatant by Sandwich ELISA

Whether an assay system for assaying the N protein may be attained or not was tested by carrying out sandwich ELISA using the recombinant N protein and virus culture supernatant. The ELISA was carried out as follows: That is, each monoclonal antibody was diluted with PBS7.4 to a concentration of 5 μg/ml, and the antibody solution was added to each well of an ELISA plate produced by FALCON

in an amount of 50 μl per well, followed by leaving the ELISA plate to stand at 4°C overnight to coat the well. Then 150 μl/well of 1% BSA-PBS7.4 was added to each well, and the plate was left to stand at 37°C for 1 hour to carry out masking. Each well was washed 3 times with a washing buffer (PBS7.4 containing 0.05% Tween 20), and then the recombinant N protein and the virus culture supernatant were added to each well in an amount of 50 μl/well, followed by allowing reaction at 37°C for 1 hour. The recombinant N protein was used at a concentration of 20 ng/ml, and the culture supernatant was used as it is or after dilution with the washing buffer. The culture supernatant of the cells not infected with the virus was used as a negative control. Then each monoclonal antibody from each hybridoma culture supernatant described in Example 1 was purified by using an anti-mouse immunoglobulin affinity column and pooled, followed by labeling of the monoclonal antibody with alkaline phosphatase. The obtained labeled antibody was added to each well in an amount of 50 μl/well, and reaction was allowed to occur at 37°C for 1 hour. After washing each well 3 times with the washing buffer, the substrate *p*-nitrophenyl phosphate (p-NPP) was added in an amount of 50 μl/well, and the resulting mixture was left to stand at room temperature for 15 minutes. The wells were visually observed and absorbance at a wavelength of 405 nm was measured. As shown in Table 1, it was confirmed that detection of N protein may be attained with any of the monoclonal antibodies used in this Example.

【0057】

【Table 1】

Antibody of the Present Invention	Sandwich ELISA Visual Observation		Sandwich ELISA A405	
	Virus Culture Supernatant	Control Culture Supernatant	Virus Culture Supernatant*	Recombinant N Protein
rSN-18	+	-	0.62	0.46
rSN-122	+	-	0.80	0.99
rSN-150	+	-	0.90	1.24
E2CT-38	-	-	0.05	0.10

*: used after 4-fold dilution

【0058】

Reference Example 4 Preparation of Alkaline Phosphatase-labeled Anti-SARS Virus Monoclonal Antibody

Each of the anti-SARS virus monoclonal antibodies prepared in Example 1 was reacted with 2-iminothiolane hydrochloric acid salt (produced by ALDRICH), thereby introducing thiol groups to the monoclonal antibody.

Then alkaline phosphatase to which maleimide groups were introduced and each of the above-described antibodies to which thiol groups were introduced were reacted, and the product was subjected to gel filtration to obtain purified alkaline phosphatase-labeled anti-SARS virus monoclonal antibodies.

【0059】

Example 4 Measurement by Sandwich ELISA Using Alkaline Phosphatase-labeled Anti-SARS Virus Monoclonal Antibody

The following sandwich ELISA was performed using the recombinant N protein and inactivated virus culture supernatant obtained by heating the culture supernatant at 56°C for 90 minutes.

Each monoclonal antibody alone or a mixture thereof was diluted to a concentration of 10 to 15 μg/ml with phosphate buffer 7.5, and was placed in the wells of an IMMUNOMODULE MAXISORP plate produced by NUNC in an amount of 100 μl/well, followed by leaving the plate to stand overnight at 4°C to immobilize the antibody. Each well was then washed 3 times with a washing buffer (TBS (Tris-buffered physiological saline) containing 0.02% Triton X-100, pH7.2), and 1% BSA-phosphate buffer 7.4 was placed in each well in an amount of 250 μl/well. The resulting plate was left to stand overnight at 37°C to carry out blocking, thereby obtaining an antibody-immobilized plate. After washing the antibody-immobilized plate 3 times with the washing buffer, the recombinant N protein (1.0

ng/ml) or the virus culture supernatant (100 µl/well) diluted with a reaction solution (PBS containing 1% BSA, pH7.5) was placed in each well, and the resulting mixture was allowed to react at room temperature (25°C) for 1 hour. A culture supernatant of the cells not infected with the virus was used as a negative control. After washing the plate 4 times with the washing buffer, the labeled antibody alone or a mixture of antibodies prepared in Reference Example 4, at a concentration of 1.0 to 5.0 µg/ml was placed in each well in an amount of 100 µl/well, followed by allowing the mixture to react at room temperature (25°C) for 1 hour. After washing the plate 4 times with the washing buffer, a substrate *p*-nitrophenyl phosphate (*p*-NPP) was placed in each well in an amount of 100 µl/well, and the resulting mixture was left to stand at room temperature for 30 to 60 minutes, followed by measurement of the absorbance at a wavelength of 405 nm. The results of the measurements of the absorbance obtained for the recombinant N protein and the virus culture supernatant are shown in Tables 2a and 2b, respectively. As shown in Table 2a, it was confirmed that detection of the recombinant N protein may be attained by any of the monoclonal antibodies, although the reactivities varies depending on the combination of the antibodies. As shown in Table 2b, the reactivities substantially the same as those for the recombinant N protein were observed for the virus culture supernatant.

【0060】

【Table 2a】

Table 2a

Labeled Antibody	Immobilized Antibody				
	rSN-122	rSN-150	rSN-18	rSN-21-2	rSN-29
rSN-122	0.029	0.416	0.253	0.429	0.439
rSN-150	0.231	0.078	0.121	0.137	0.127
rSN-18	0.140	0.136	0.071	0.067	0.101
rSN-21-2	0.255	0.162	0.127	0.042	0.052
rSN-29	0.240	0.140	0.117	0.028	0.027

【0061】

【Table 2b】

Table 2b

Labeled Antibody	Immobilized Antibody				
	rSN-122	rSN-150	rSN-18	rSN-21-2	rSN-29
rSN-122	0.069	2.339	0.197	1.697	2.264
rSN-150	1.801	0.032	0.086	0.916	1.099
rSN-18	0.067	0.080	0.030	0.049	0.059
rSN-21-2	1.907	1.194	0.076	0.062	0.043
rSN-29	2.104	1.260	0.084	0.040	0.030

【0062】

Example 5 Measurement by Immunochromatography

Rapid detection of the N protein by immunochromatography using the recombinant N protein or the virus culture supernatant inactivated by heat treatment at 56°C for 90 minutes was confirmed. An immunoassay device 1 for immunochromatography, shown in Fig. 4 was prepared as follows:

On one end of a nitrocellulose membrane 2 (5 mm x 50 mm), a developer-supplying zone 3 having a substrate zone 7 prepared by spotting a 20 mg/ml solution of sodium 5-bromo-4-chloro-3-indolyl phosphate (BCIP·Na) as a substrate on a water-absorptive non-woven fabric and dried, was formed, and a water-absorptive absorption pad (developer-absorption zone 5) was formed on the other end of the membrane. A detection zone 6 was formed at a region downstream, in the direction of the liquid transportation, of the labeled reagent zone 4 (sample-spotting zone 8) in the membrane part. The detection zone 6 was prepared by spotting a solution of the monoclonal antibody (1 mg/ml) shown in Table 3a or 3b in the form of a line and drying the solution. On the nitrocellulose membrane after blocking with PBS containing BSA or without the blocking, the labeled reagent zone 4 prepared by spotting a solution of a single or two types of the alkaline phosphatase-labeled monoclonal antibody (35 ng/pad) shown in Table 3a or 3b on a water-absorptive non-woven fabric and drying the solution was mounted.

A sample 9 (25 to 30 μl) prepared by diluting the recombinant N protein or the culture supernatant with Tris-buffered physiological saline containing 3% BSA

(sample treatment solution) was spotted on the sample-spotting zone 8 formed on the labeled reagent zone 4, of the thus prepared immunoassay device 1. Then 300 μl of a developer 10 was dropped on the developer-supply zone 3 and the sample and the substrate were allowed to develop in the nitrocellulose membrane, and 15 minutes later, emergence of a line at the detection zone 6 was checked. The results are shown in Table 3a. As shown in Table 3a, although the reactivities varied depending on the combination of the antibodies, the recombinant N protein was able to be detected in a reaction time of 15 minutes. On the other hand, based on the results shown in Tables 2a, 2b and Table 3a, combinations of the immobilized antibody and the labeled antibody were selected, which showed high reactivities. Using the selected combinations, assay of the virus culture supernatant was carried out. As a result, the N protein in the virus culture supernatant was able to be detected at high dilution factors. The results are shown in Table 3b.

【0063】

【Table 3a】

Table 3a

Immobilized Antibody	Labeled Antibody		
	rSN-150	rSN-122	rSN-18
rSN-150	-	4w	2
rSN-122	3	3w	4
rSN-18	2	4	-

Values (color intensity) in the table were determined by visual observation of the color intensity of the detection line at 15 minutes from the beginning of the reaction. (4>4w>3>3w>2>2w>1, -: line not detected)

【0064】

【Table 3b】

Table 3b

Labeled Antibody	Immobilized Antibody			
	rsN-150	rsN-122	rSN-21-2	rSN-29
rSN-122	1500	-	20000	15000
rSN-150	-	1500	1500	1500
rSN-21-2	1500	30000	-	-
rSN-29	3000	30000	-	-
rSN-122 + rSN-150	1000	-	>3000	>3000
rSN-122 + rSN-18	1000	-	>3000	>3000

The values indicate dilution factors at which the culture supernatant was able to be detected.

- indicates not determined.

>3000 indicates that a dilution factor of 3000-fold or more was able to be detected.

【0065】

Reference Example 5 Synthesis of N5 Peptide and Preparation of KLH Conjugate

A peptide corresponding to the amino acids 244-260 of the SARS nucleoprotein (GQTVTKKSAAEASKKPRC) shown in SEQ ID NO:3 in SEQUENCE LISTING was synthesized by the Fmoc method with a peptide synthesizer produced by SHIMADZU CORPORATION (PSSM-8). N5 peptide was synthesized by the method described in the instruction of the synthesizer. The synthesized peptide was conjugated to keyhole limpet hemocyanin (KLH) in accordance with a conventional method to obtain a KLH conjugate.

【0066】

Example 6 Establishment of Anti-N Protein Monoclonal Antibody Using N5 Peptide Antigen

A monoclonal antibody against the N protein was prepared by immunizing a mouse with the N5 peptide-KLH conjugate prepared in Reference Example 5, and

fusing lymphocytes from the spleen of the mouse and myeloma cells. The preparation was carried out by repeating the method described in Example 1. After screening, a hybridoma SN5-25 producing an anti-N protein monoclonal antibody was established. The monoclonal antibody obtained from this hybridoma was named SN5-25.

【0067】

Example 7 Assay by Sandwich ELISA Using Alkaline Phosphatase-labeled Anti-SARS Virus Monoclonal Antibody

As in Reference Example 4, alkaline phosphatase-labeled anti-SARS virus monoclonal antibodies shown in Table 4 were prepared. Further, as in Example 4, antibody-immobilized plates shown in Table 4 were prepared, and assays were performed using the virus culture supernatant. The results are shown in Table 4. As a result, the N protein in the virus culture supernatant was able to be detected at high dilution factors even using the monoclonal antibodies whose antigen is the peptide corresponding to the SARS nucleoprotein (244-260).

【0068】

【Table 4】

Culture Supernatant ($TCID_{50}$/mL)	Immobilized Antibody	Labeled Antibody	Detection
3.55×10^4	SN5-25	rSN-18	+
1.77×10^4	SN5-25	rSN-18	+
1.22×10^4	SN5-25	rSN-18	+
	rSN-150	rSN-122	+
8.11×10^3	rSN-150	rSN-122	+

【Brief Description of the Drawings】

【0069】

【Fig. 1】 Fig. 1 shows a restriction map of an expression plasmid pW6A used in the present invention.

【Fig. 2】 Fig. 2 shows the results of the measurement of an expressed

recombinant protein (S-N).

【Fig. 3】 Fig. 3 shows the reactivities of the monoclonal antibodies (rSN-18 antibody, rSN-122 antibody and rSN-150 antibody) confirmed by WB.

【Fig. 4】 Fig. 4 shows the reactivities of the monoclonal antibodies (rSN-21-2 antibody, rSN-29 antibody and rSN-122 antibody) confirmed by WB.

【Fig. 5】 Fig. 5 shows a schematic cross-sectional view of the immunoassay device 1 for immunochromatography according to the present invention.

【Sequence Listing Free Text】

【0070】

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Ala Arg Met Ala Ser Gly Gly Gly Glu Thr Ala Leu Ala Leu Leu Leu
    210                 215                 220

Leu Asp Arg Leu Asn Gln Leu Glu Ser Lys Val Ser Gly Lys Gly Gln
225                 230                 235                 240

Gln Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu Ala Ser
                245                 250                 255

Lys Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Gln Tyr Asn Val Thr
            260                 265                 270

Gln Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn Phe Gly
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Asp Gln Asp Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp Pro Gln
    290                 295                 300
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Ile Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met Ser Arg
305 310 315 320

Ile Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr His Gly
325 330 335

Ala Ile Lys Leu Asp Asp Lys Asp Pro Gln Phe Lys Asp Asn Val Ile
340 345 350

Leu Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro Thr Glu
355 360 365

Pro Lys Lys Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro Leu Pro
370 375 380

Gln Arg Gln Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala Ala Asp
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Met Asp Asp Phe Ser Arg Gln Leu Gln Asn Ser Met Ser Gly Ala Ser
405 410 415

Ala Asp Ser Thr Gln Ala
420

<210> 3
<211> 18
<212> PRT
<213> Coronavirus

<400> 3

Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu Ala Ser Lys Lys Pro
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Arg Cys

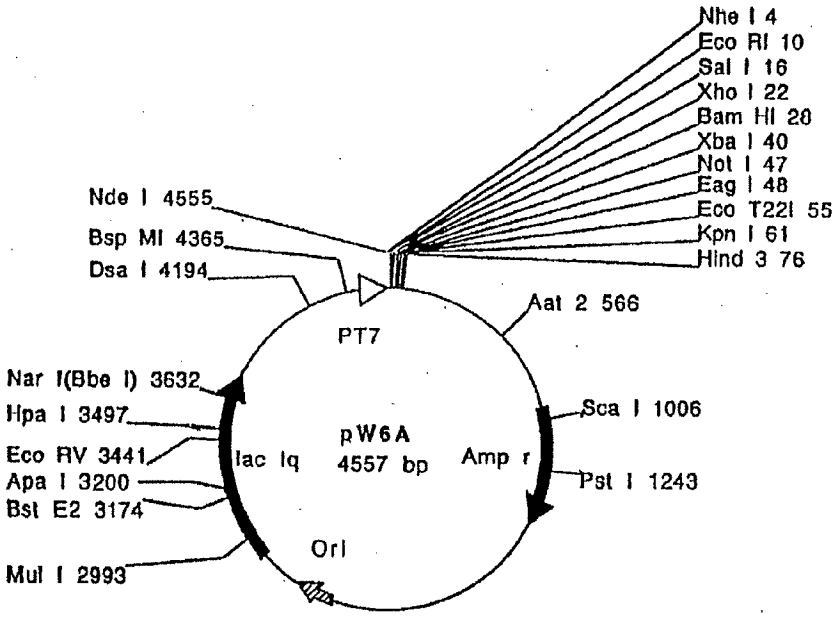
Nhe I 4
Eco RI 10
Sal I 16
Xho I 22
Bam HI 28
Xba I 40
Not I 47
Eag I 48
Eco T22I 55
Kpn I 61
Hind 3 76
Nde I 4555
Bsp MI 4365
Dsa I 4194
Aat 2 566
PT7
Nar I(Bbe I) 3632
Hpa I 3497
Eco RV 3441
Apa I 3200
Bst E2 3174
Mul I 2993
pW6A
4557 bp
lac Iq
Amp r
Sca I 1006
Pst I 1243
Ori

Fig. 1

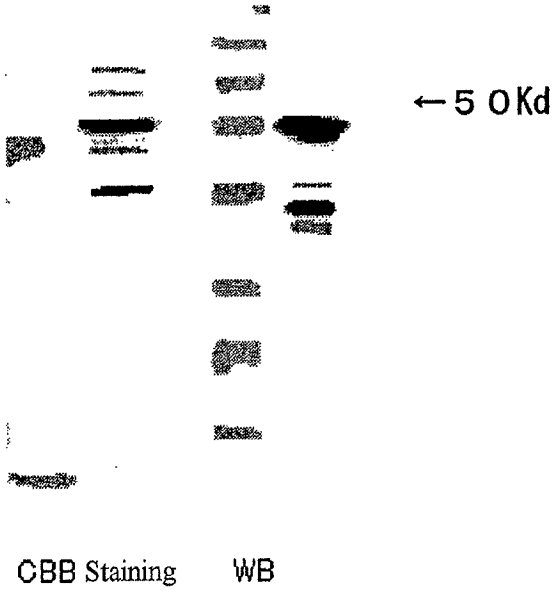
←5 0Kd
CBB Staining
WB

Fig. 2

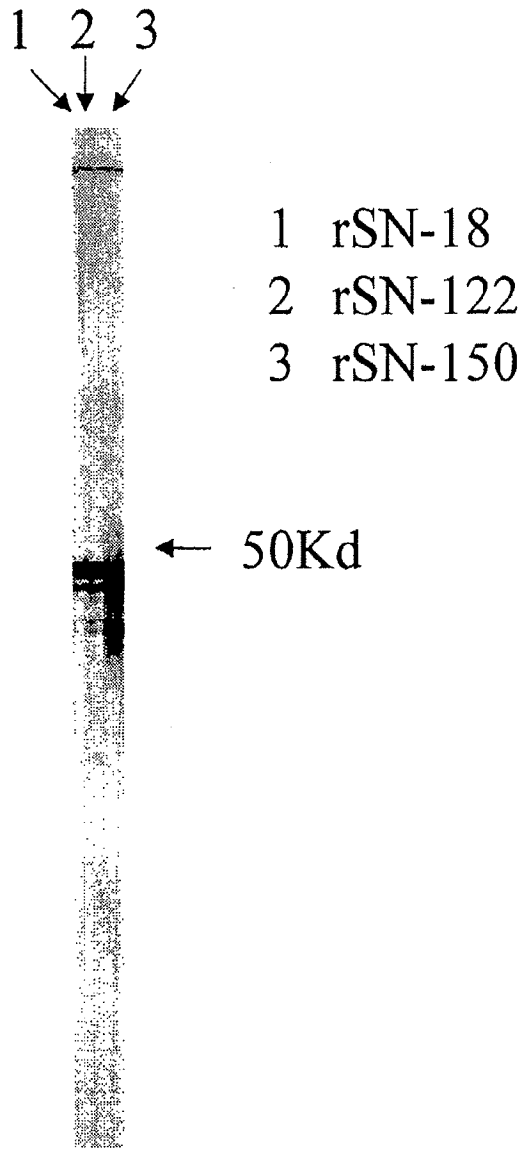

Fig. 3

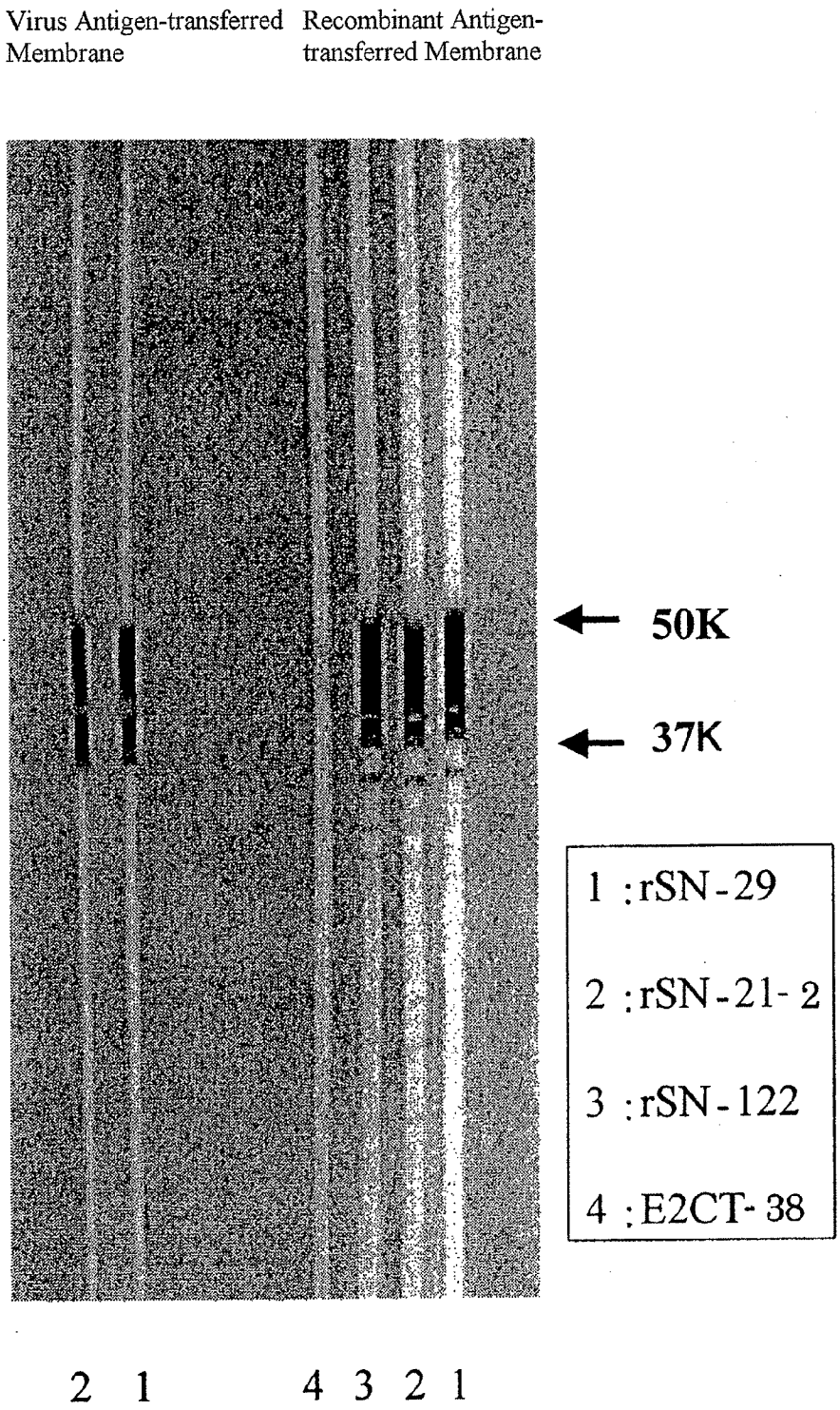
Virus Antigen-transferred Membrane
Recombinant Antigen-transferred Membrane
50K
37K
1 : rSN-29
2 : rSN-21-2
3 : rSN-122
4 : E2CT-38
2 1
4 3 2 1

Fig. 4

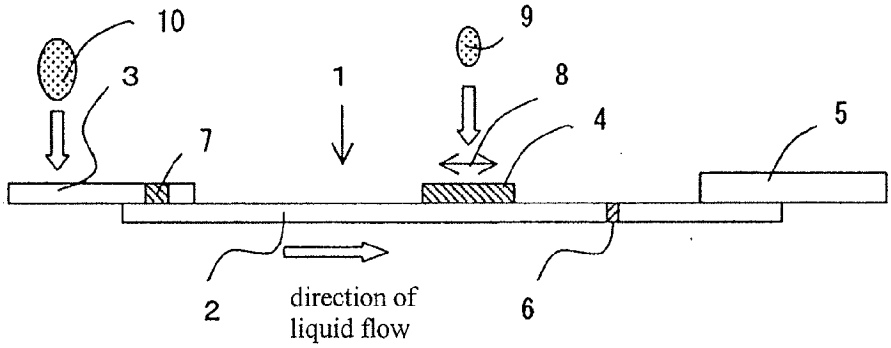

Fig. 5

【TITLE OF DOCUMENT】 ABSTRACT

【Abstract】

【Object】

To provide a monoclonal antibody against the nucleoprotein of the severe acute respiratory syndrome (SARS)-causing coronavirus, hybridoma which produces the monoclonal antibody and immunoassay reagent for SARS virus, which uses the monoclonal antibody as at least one of the immobilized antibody and labeled antibody.

【Means for Solution】

A monoclonal antibody which reacts with the nucleoprotein of SARS virus obtained by using as an immunogen the nucleoprotein of said coronavirus, which nucleoprotein is expressed by a vector in which a nucleotide sequence shown in SEQUENCE LISTING 2 is incorporated, is prepared.

【Selected Drawings】 Fig. 3